Summary Report of the EPISKIN™ *In Vitro* Assay for Assessing Dermal Corrosivity

Prepared for

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PURPOSE

This report focuses on the performance of EPISKIN™ to determine the usefulness and limitations of the assay for the identification of potential human corrosive chemicals. This report also discusses how the EPISKIN™ assay compares to the *in vivo* rabbit skin corrosivity test and to other *in vitro* corrosivity tests (Rat Skin Transcutaneous Electrical Resistance [TER], EpiDerm™ [EPI-200], and Corrositex®). The data and assessments in the European Centre for the Validation of Alternative Methods (ECVAM) formal validation study on EPISKIN™ (Barratt et al., 1998; Fentem et al., 1998) were reviewed. Additionally, an independent analysis of the performance data, based on the information provided in Fentem et al. (1998), was conducted.

EVALUATION OF REGULATORY AND SCIENTIFIC RATIONALE

EPISKIN™ is one of several *in vitro* corrosivity assays evaluated as alternatives to the *in vivo* rabbit corrosivity test by ECVAM in a formal validation study (Fentem et al., 1998). EPISKIN™ is a three-dimensional human skin model that measures cell viability. Because it is a human skin model, it may be more relevant to assessing human skin corrosivity potential than a test based on skin from another species. Also, the mode of application (topical) of the test material mimics the route of human exposure.

EPISKIN™ has been endorsed by the ECVAM Scientific Advisory Committee for use in corrosivity testing in Europe (Balls and Corcelle, 1998) and EPISKIN™ has also been evaluated and endorsed for its intended use by the European Commission Scientific Committee for Cosmetic Products and Non-food Products (SCCNFP) (Anon., 1999). This method has been adopted for regulatory use within the European Union (EU) by the European Commission (EU, 2000).

EVALUATION OF THE TEST METHOD

A standard kit contains media, reagents, and 12 epidermis units. The epidermis units provided in the test kit are comprised of a reconstructed epidermis and a functional stratum corneum. For use in corrosivity testing, the test material (liquids: 50 µL; solids: 20 mg) is topically applied to an epidermis unit for 3, 60, and 240 minutes. Per test compound, one epidermis unit is needed for each of the three test periods. Cell viability is assessed by measuring mitochondrial activity using the MTT (a tetrazolium salt) assay. A 35% decrease in cell viability is used to indicate a potential for human corrosivity. The scientific and mechanistic basis of the test and the rationale for using a 35% decrease in cell viability as the criterion for identifying potential human corrosivity were not discussed by Fentem et al. (1998). However, mechanistically, corrosivity is associated with cell death.

EVALUATION OF TEST METHOD DATA QUALITY

Only limited validation test data are available on EPISKIN™. In the single published validation study by Fentem et al. (1998), ECVAM evaluated 60 chemicals. The chemical selection procedure was described in sufficient detail by Barratt et al. (1998). The main criterion for including chemicals in the study was that their corrosivity classification (C= corrosive; NC = noncorrosive) was based on unequivocal animal data (Barratt et al., 1998). The ECVAM validation chemical test set

included organic acids (6C/5NC), organic bases (7C/3NC), neutral organics (9NC), phenols (2C/3NC), inorganic acids (6C/1 NC), inorganic bases (2C/2NC), inorganic salts (1C/2NC), electrophiles (3C/5NC), and soaps/surfactants (3NC). Despite the small numbers of chemicals in some categories, ECVAM concluded that the set of test chemicals represented the best possible group for evaluating the performance characteristics of the *in vitro* assays, given the limited availability of unequivocal animal data (Barratt et al., 1998).

Each chemical was tested three times by each of three different laboratories. The tests were stated to have been conducted in the "spirit" of GLP (Fentem et al., 1998). A formal audit of the ECVAM data by a Quality Assurance Unit was not conducted; however, it was stated that all data submitted by the participating laboratories were verified against the original data sheets by ECVAM staff on at least three separate occasions.

EVALUATION OF TEST METHOD PERFORMANCE

For this summary report, an analysis was conducted, similar to the performance analysis conducted for the ICCVAM Peer Review of Corrositex®; the current analysis evaluated the performance characteristics of the EPISKIN™ assay against the corresponding *in vivo* rabbit corrosivity data. The database used in the EPISKIN™ evaluation consisted of data from the ECVAM validation study only; other data were not located. For ease of comparison, chemicals evaluated in the EPISKIN™ assay were classified into the same chemical and product class designations used in the Corrositex® evaluation. A weight-of-evidence approach was used for classifying discordant results within or between laboratories; in instances where discordant results could not be resolved (i.e., there was an equal number of positive and negative calls), the chemical was eliminated from inclusion in the performance calculations.

Based on the database of 60 chemicals and chemical mixtures used in the validation study (**Table 2.1**), EPISKIN™ had an accuracy of 83% (50/60 chemicals or chemical mixtures), a sensitivity of 82% (23/28 chemicals or chemical mixtures), a specificity of 84% (27/32 chemicals or chemical mixtures), a false positive rate of 16% (5/32 chemicals or chemical mixtures), and a false negative rate of 18% (5/28 chemicals or chemical mixtures). Furthermore, EPISKIN™ was able to distinguish between known R35/I and R34/II & III chemicals[1]. Based on these data, which met pre-study acceptance criteria of no more than 20% false negatives and 20% false positives, the ECVAM study Management Team concluded that EPISKIN™ was valid for use as a replacement for the *in vivo* rabbit skin test for distinguishing between corrosive and noncorrosive chemicals for all of the chemical classes studied (Fentem et al., 1998; Balls and Corcelle, 1998). Because of the relatively small numbers of chemicals evaluated in some chemical classes (i.e., cleaners and detergents), definitive conclusions as to the adequacy of

[1]UN packing group classifications I, II, and III are assigned based on the capacity of a chemical, when tested on the intact skin of rabbits, to produce skin corrosion following exposure intervals of 3 minutes, 1 hour, or 4 hours, respectively (Fentem et al., 1998). EU regulations require classification of chemicals according to certain risk phases, such as those assigned based on whether the chemical causes corrosion following a 3-minute application (R35 – “causes severe burns”; analogous to packing group I) or 4 hours (R34 – “causes burns”; analogous to packing groups II and III) (Barratt et al., 1998; Fentem et al., 1998).

EPISKIN™ for some classes of chemicals were difficult to make with a high degree of confidence. Additionally, no assessment could be made with respect to mixtures. However, it was stated that taking into account the relative simplicity of the mechanism of action of corrosives, this method would be generally applicable across all chemical classes (Fentem et al., 1998).

EVALUATION OF TEST METHOD RELIABILITY (REPEATABILITY/ REPRODUCIBILITY)

The inter- and intra-laboratory reliability of EPISKIN™ was evaluated in the ECVAM validation study (Fentem et al., 1998). In each laboratory, each chemical was tested three times using three different batches of EPISKIN™. Intra- and inter-laboratory reliability was evaluated using a relative mean square diagram (determined using a two-way ANOVA with laboratory and experiments as factors), scatter diagrams to assess the possibility of divergence between results obtained in different laboratories, and range diagrams to summarize the overall performance of the tests. Of the 60 chemicals tested, 42 gave the same corrosivity classification in all three experiments in all three laboratories. In seven cases, the median results for the three laboratories gave identical predictions. In only three cases did one laboratory give results that were consistently in a different classification category than those from the other laboratories. In an additional three cases, the median result from one laboratory was in a different category than those from the other laboratories, and in five cases, chemicals gave results that crossed the classification boundaries in more than one laboratory. Although there were differences for some chemicals in calls between experiments within and between laboratories, ECVAM concluded that EPISKIN™ met the criteria agreed by the Management Team concerning acceptable intra- and inter-laboratory reproducibility (Fentem et al., 1998). Due to the lack of quantitative data, by experiment and laboratory, for individual chemicals in the published studies, no independent evaluation of repeatability or reproducibility for EPISKIN™ could be conducted. However, after reviewing the intra- and inter-laboratory evaluations conducted by ECVAM, it was concluded by NICEATM that the analyses were appropriate and that the conclusions were accurate.

Table 2.1 Performance of the EPISKIN™ Assay in Predicting Corrosivity/Noncorrosivity Compared to *In Vivo* Findings (Fentem et al., 1998)

Chemical or Product Class	Number of Chemicals	Accuracy		Sensitivity		Specificity	
		%	Number	%	Number	%	Number
Overall	**60**	**83**	**(50/60)**	**82**	**(23/28)**	**84**	**(27/32)**
Organic and Inorganic Acids and Bases[1]	**41**	**78**	**(32/41)**	**81**	**(21/26)**	**73**	**(11/15)**
Organic and Inorganic Bases and Base Mixtures[2]	**14**	**64**	**(9/14)**	**60**	**(6/10)**	**75**	**(3/4)**
Organic and Inorganic Acids and Acid Mixtures	**20**	**85**	**(17/20)**	**100**	**(11/11)**	**67**	**(6/9)**
Amines	**10**	**60**	**(6/10)**	**57**	**(4/7)**	**67**	**(2/3)**
Inorganic Bases and Base Mixtures	**4**	**75**	**(3/4)**	**67**	**(2/3)**	**100**	**(1/1)**
Acid Derivatives	**7**	**86**	**(6/7)**	**80**	**(4/5)**	**100**	**(2/2)**
Surfactants	**5**	**80**	**(4/5)**	**NA**	**(0/0)**	**80**	**(4/5)**
Industrial Chemicals	**10**	**100**	**(10/10)**	**100**	**(1/1)**	**100**	**(9/9)**
Cleaners and Detergents	**1**	**100**	**(1/1)**	**NA**	**(0/0)**	**100**	**(1/1)**

[1] This chemical class includes chemicals from the following chemical classes: organic and inorganic bases and base mixtures, organic and inorganic acids and acid mixture, and acid derivatives

[2] This chemical class includes amines, inorganic bases, and base mixtures.

OTHER SCIENTIFIC REVIEWS

In March 1999, a search of the open literature was conducted to locate additional EPISKIN™ studies. Six databases (Medline, Toxline, Embase, Biosis, Caba, and LifeSci) were searched using the key terms "Episkin", and "Epi" within one word of "skin". The search found no additional relevant studies conducted with EPISKIN™. In May 2001, another search was conducted to locate additional EPISKIN™ studies. Four databases (PubMed, Web of Science, Toxline, and Current Contents Connect) were searched using the same search strategy and no additional relevant studies were found.

OTHER CONSIDERATIONS

The EPISKIN™ kit contains all of the necessary materials to conduct the test and does not require additional preparation. No animals are used in this test. ECVAM concluded that, compared to the *in vivo* test method, EPISKIN™ costs less to perform (Fentem et al., 1998). The cost for conducting EPISKIN™ is reported by L'OREAL Recherche (e-mail communication from Odile de Silva, L'OREAL Recherche) to be approximately $450 per kit (**Table 2.2**). When compared to other *in vitro* corrosivity test methods, the cost of EPISKIN™ is stated to be greater than that of the Corrositexâ and EpiDerm™ (EPI-200) assays and somewhat less than the Rat Skin TER (Fentem et al., 1998).). The EPISKIN™ human skin model is commercially available from EPISKIN SNC, Lyon, France, a wholly owned subsidiary of L'OREAL. The time needed to conduct the EPISKIN™ assay is greater than the Corrositex® assay, comparable to the EpiDerm™ (EPI-200) assay, and less than the Rat Skin TER assay.

RELATED ISSUES

Refinement, Reduction, and Replacement

Since the method is designed as a replacement for animals, EPISKIN™ would clearly reduce the requirement for animal testing for corrosivity. Therefore, it has the potential to eliminate the use of animals for the determination of corrosivity. If used in an integrated approach, EPISKIN™ provides for reduction and refinement of animal use.

Comparison to Other *In Vitro* Assays

General comparative information on the TER, EPISKIN™, and Corrositexâ assays is provided in **Tables 2.2** through **2.5**.

Table 2.2 General Comparison of the Rat Skin TER, EPISKIN™, EpiDerm™ (EPI-200), and Corrositex® Assays

	Rat Skin TER	EPISKIN™ (prediction model B)	EpiDerm™ (EPI-200) (prediction model 2)	Corrositex®
Test Method Description	Acceptable	Acceptable	Acceptable	Acceptable
Adequacy/Completeness of Protocol	Acceptable	Acceptable	Acceptable	Acceptable
Usefulness for Assessing Corrosivity/Non-corrosivity	Acceptable (Botham et al., 1992; 1995; Fentem et al., 1998)	Acceptable (Fentem et al., 1998)	Acceptable (Liebsch et al., 2000)	Acceptable (ICCVAM, 1999)
Usefulness for Determining Packing Groups	Not Acceptable (Fentem et al., 1998)	Can group as UN packing group II/III or I (Fentem et al., 1998)[a]	Not Acceptable (Liebsch et al., 2000)	Acceptable (ICCVAM, 1999)
Repeatability and Reproducibility	Acceptable (Botham et al., 1992; 1995; Fentem et al., 1998)	Acceptable (Fentem et al., 1998)	Acceptable (Liebsch et al., 2000)	Acceptable (Fentem et al., 1998; ICCVAM, 1999)
Animal Use Refinement, Reduction, and Replacement Considerations	Refines and reduces animal use when used as a stand-alone test or in an integrated testing strategy.	Replaces animal use when used as a stand-alone test. Refines and reduces animal use when used in an integrated testing strategy.	Refines and reduces animal use when used in an integrated testing strategy.	Replaces animal use when used as a stand-alone test. Refines and reduces animal use when used in an integrated testing strategy.
Cost	~$500-850/test	~$450/test kit[b]	~$200/test chemical	~$300/test chemical
Study Duration	2 work-days	1 work-day	1 work-day	≤ 4 hr/chemical

[a] Since the performance of EPISKINä was not assessed for distinguishing between UN packing groups II and III, all R34 classifications would be conservatively classified as UN packing group II.

[b] One to three chemicals may be tested per test kit; however, it is recommended by the supplier that each test chemical be assayed using 3 different skin batches/kits which equates to a total cost of ~$430/ test chemical.

Table 2.3 General Comparison of the Rat Skin TER, EPISKIN™, EpiDerm™ (EPI-200), and Corrositex® Assays Based on a Weight-of-Evidence Approach[a] by Chemical using Data from the ECVAM and other Validation Studies (Fentem et al., 1998; ICCVAM, 1999; Liebsch et al., 2000)

	Rat Skin TER	EPISKIN™	EpiDerm™ (EPI-200) (prediction model 2)	Corrositex®
Number of Chemicals	122	60	24	163
Overall Sensitivity[b]	94% (51/54)	82% (23/28)	92% (11/12)	85% (76/89)
Overall Specificity[c]	71% (48/68)	84% (27/32)	83% (10/12)	70% (52/74)
Overall Accuracy[d]	81% (99/122)	83% (50/60)	92% (22/24)	79% (128/163)
False Positive Rate	29% (20/68)	16% (5/32)	17% (2/12)	30% (22/74)
False Negative Rate	6% (3/54)	18% (5/28)	8% (1/12)	15% (13/89)
Test Chemical Inter-laboratory Coefficient of Variation	34.7[e] 3.8-322[f] 120[g]	11.3[e] 3.9-148.8[f] 20[g]	12.3[e] 0.9-51.2[f] 144[g]	30.3[e] 7.7-252.5[f] 180[g]

[a] A chemical is first classified as positive or negative for corrosivity within each laboratory based on the majority of test results obtained (when replicate testing was conducted). Next, the chemical is classified as positive or negative for corrosivity based on the majority of test results obtained in multiple laboratories (when multiple laboratory studies were conducted). In instances where discordant results could not be resolved (i.e., there was an equal number of positive and negative calls within or across laboratories), the chemical was eliminated from inclusion in the performance calculations.

[b] Sensitivity is defined as the proportion of all positive chemicals that are correctly classified as positive in a test.

[c] Specificity is defined as the proportion of all negative chemicals that are correctly classified as negative in a test.

[d] Accuracy (concordance) is defined as the proportion of correct outcomes of a method.

[e] Median values

[f] Range of values

[g] The total number of independent values, which is calculated as the number of chemicals tested multiplied by the number of participating laboratories.

Table 2.4 General comparison of the Rat Skin TER, EPISKIN™, and EpiDerm™ (EPI-200) assays from independent test results in the ECVAM validation studies (Fentem et al., 1998; Liebsch et al., 2000)

	Rat Skin TER	EPISKIN™ (prediction model B)	EpiDerm™ (EPI-200) (prediction
Number of Chemicals Tested in ECVAM Validation Study	60 (Fentem et al., 1998)	60/24[a] (Fentem et al., 1998)	24 (Liebsch et al., 2000)
Sensitivity[b]	88% (140/159)	83% (201/243) / 88% (87/99)	88% (63/72)
Specificity[b]	72% (142/196)	80% (237/297) / 79% (92/117)	86% (62/72)
Accuracy[b]	79% (282/355)[c]	81% (438/540) / 83% (179/216)	87% (125/144)
False Positive Rate[b]	28% (54/196)	20% (60/297) / 21% (25/117)	14% (10/72)
False Negative Rate[b]	12% (19/159)	17% (42/243) / 12% (12/99)	13% (9/72)
Number of Trials[f]	355	540 / 216	144
Test Chemical Inter-laboratory Coefficient of Variation	34.7[d] 10-322[e] 360[f]	30.2[d] 7.7-252.5[e] 540[f]	12.3[d] 0.9-51.2[e] 144[f]

[a] The first numbers for accuracy, sensitivity, specificity, false positive rate, and false negative rate correspond to the 60 chemicals tested in the ECVAM Skin Corrosivity Test using EPISKIN™ (Barratt et al., 1998; Fentem et al., 1998); the latter values correspond to a direct comparison of EpiDerm™ (EPI-200) and EPISKIN™ for the same 24 materials tested in both systems (Liebsch et al., 2000).

[b] Sensitivity is defined as the proportion of all positive chemicals that are correctly classified as positive in a test. Specificity is defined as the proportion of all negative chemicals that are correctly classified as negative in a test. Accuracy (concordance) is defined as the proportion of correct outcomes of a method. False positive rate is defined as the proportion of all negative chemicals or chemical mixtures that are falsely identified as positive. False negative rate is defined as the proportion of all positive chemicals or chemical mixtures that are falsely identified as negative.

[c] The percentages are based on the number of correct trials among the total number of trials (i.e., independent tests) provided in parenthesis.

[d] Median values

[e] Range of values

[f] The total number of trials conducted in the validation study minus the non-qualified (NQ) results. This number is equal to the number of chemicals multiplied by the number of participating laboratories multiplied by the number of replicate tests.

Table 2.5 Classification Results from the ECVAM Validation Studies of Rat Skin TER, EPISKIN™, and EpiDerm™ (EPI-200) Assays as Compared to the *In Vivo* Classification (Fentem et al., 1998; Liebsch et al., 2000)

No.[a]	Chemical	Type	*In Vivo*	Rat Skin TER	EPISKIN™ [b]	EpiDerm™ (EPI-200)
1	Hexanoic acid	ORGAC	R34/II&III	R35	R35	N/A
29	65/35 Octanoic/decanoic acid	ORGAC	R34/II&III	R34	R35	N/A
36	2-Methylbutyric acid	ORGAC	R34/II&III	R35	R34	N/A
40	Octanoic acid (caprylic acid)	ORGAC	R34/II&III	R35	R34/C	C
47	60/40 Octanoic/decanoic acids	ORGAC	R34/II&III	R34	R34/C	C
50	55/45 Octanoic/decanoic acids	ORGAC	R34/II&III	R35	R34	N/A
7	3,3'-Dithiodipropionic acid	ORGAC	NC	NC	NC	N/A
12	Dodecanoic acid (lauric acid)	ORGAC	NC	NC	NC	NC
26	Isotearic acid	ORGAC	NC	NC	NC	NC
34	70/30 Oleine/octanoic acid	ORGAC	NC	NC	NC	N/A
58	10-Undecenoic acid	ORGAC	NC	NC	R34	N/A
2	1,2-Diaminopropane	ORGBA	R35/I	R35	R34/C	C
15	Dimethyldipropylenetriamine	ORGBA	R35/I	R35	R34/C	C
38	Tallow amine	ORGBA	R35/II	2R34/2NC/2NQ	NC	N/A
55	1-(2-Aminoethyl)piperazine	ORGBA	R34/II	R35	NC	N/A
13	3-Methoxypropylamine	ORGBA	R34/II&III	R35	R34	N/A
17	Dimethylisopropylamine	ORGBA	R34/II&III	R35	R34/C	C
45	n-Heptylamine	ORGBA	R34/II&III	R35	NC	C
10	2,4-Xylidine (2,4-Dimethylaniline)	ORGBA	NC	R34	R34	N/A
35	Hydrogenated tallow amine	ORGBA	NC	NC	NC	NC
59	4-Amino-1,2,4-triazole	ORGBA	NC	NC	NC	NC
8	Isopropanol	NORG	NC	NC	NC	N/A
11	2-Phenylethanol	NORG	NC	NC	NC	N/A
16	Methyl trimethylacetate (referred to as Methyl 2,2-dimethylpropanoate in EpiDerm™ (EPI-200)	NORG	NC	NC	NC	C
19	Tetrachloroethylene	NORG	NC	NC	NC	NC
22	n-Butyl propionate	NORG	NC	NC	NC	N/A
27	Methyl palmitate	NORG	NC	NC	NC	N/A
44	Benzyl acetone	NORG	NC	NC	NC	NC
51	Methyl laurate	NORG	NC	NC	NC	N/A
56	1,9-Decadiene	NORG	NC	NC	NC	NC
3	Carvacrol	PHEN	R34/II&III	R34	R34	N/A
23	2-tert-Butylphenol	PHEN	R34/II&III	R35	R34/C	C
9	*o*-Methoxyphenol (Guaiacol)	PHEN	NC	NC	R34	N/A
30	4,4-Methylene-bis-(2,6-di-tert-butylphenol)	PHEN	NC	NC	NC	N/A
49	Eugenol	PHEN	NC	NC	NC	NC

Table 2.5 (continued)

No.[a]	Chemical	Type	*In Vivo*	Rat Skin TER	EPISKIN™ [b]	EpiDerm™ (EPI-200)
4	Boron trifluoride dihydrate	INORGAC	R35/I	R35	R35/C	C
28	Phosphorus tribromide	INORGAC	R35/I	R35	R35/C	C
32	Phosphorus pentachloride	INORGAC	R35/I	R35	R34	N/A
25	Sulfuric acid (10% wt.)	INORGAC	R34/II&III	R34	R34	N/A
57	Phosphoric acid	INORGAC	R34/II	R35	R34	N/A
43	Hydrochloric acid (14.4% wt)	INORGAC	R34/II&III	R35	R34	N/A
53	Sulfamic acid	INORGAC	NC	R34	R34/C	C
18	Potassium hydroxide (10% aq.)	INORGBA	R34/II&III	R35	R34/C	C
42	2-Mercaptoethanol,Na salt (45%aq.)	INORGBA	R34/II&III	R35	NC	N/A
21	Potassium hydroxide (5% aq.)	INORGBA	NC	R35	R34	N/A
24	Sodium carbonate (50% aq.)	INORGBA	NC	R34	NC	NC
20	Ferric [iron (III)] chloride	INORGSAL	R34/II	R35	R34	N/A
52	Sodium bicarbonate	INORGSAL	NC	R34	NC	N/A
54	Sodium bisulfite	INORGSAL	NC	3R34/3NC	NC	N/A
5	Methacrolein	ELECTRO	R34/II&III	NC	R34/C	NC
14	Allyl bromide	ELECTRO	R34/II&III	R35	R34	N/A
48	Glycol bromoacetate (85%)	ELECTRO	R34/II&III	NC	R34/C	C
6	Phenethyl bromide	ELECTRO	NC	NC	NC	N/A
31	2-Bromobutane	ELECTRO	NC	3R34/3R35	NC	N/A
33	4-(Methylthio)-benzaldehyde	ELECTRO	NC	NC	NC	N/A
39	2-Ethoxyethyl methacrylate	ELECTRO	NC	NC	NC	N/A
46	Cinnamaldehyde	ELECTRO	NC	NC	NC	N/A
37	Sodium undecylenate (33% aq.)	SOAP	NC	R35	R34	N/A
41	20/80 Coconut/palm soap	SOAP	NC	NC	NC	N/A
60	Sodium lauryl sulfate (20% aq.)	SOAP	NC	R35	NC	NC

Overall corrosivity classifications were determined by the majority of the reported results obtained from each assay. If results do not show a majority, a definitive classification could not be determined.

Definitions are as follows: C = Corrosive; NC = Noncorrosive; R34 is equivalent to packing groups II and/or III; R35 is equivalent of packing group I, except for tallow amine (R35/II); NQ = Non-qualified; N/A = Not applicable because not tested; ORGAC = Organic acid; ORGBA = Organic base; NORG = Neutral organics; PHEN = phenol; INORGAC = Inorganic acid; INORGBA = Inorganic base; INORGSAL = Inorganic salt; ELECTRO = Electrophile; SOAP = Soap surfactant

[a] Number assigned each chemical by the ECVAM Management Team.

[b] For EPISKIN™, prediction model B was the more complex prediction model and was the only model considered in detail by the ECVAM Management Team (Fentem et al., 1998).

SUMMARY CONCLUSIONS AND RECOMMENDATIONS

ECVAM concluded that EPISKIN™ was an *in vitro* replacement assay for *in vivo* corrosivity testing. Although there were differences for some chemicals in calls between experiments within and between laboratories, ECVAM concluded that EPISKIN™ was both reliable and reproducible. NICEATM concurs with that conclusion. For some chemical or product classes (e.g., industrial chemicals, cleaners and detergents), the small number of chemicals and/or the unbalanced distribution of corrosive and noncorrosive chemicals does not allow accurate conclusions to be made on the performance of EPISKIN™ for those chemical classes.

The two major questions to be addressed for *in vitro* corrosivity assays are:

1. Has the assay been evaluated sufficiently and is its performance satisfactory to support the proposed use for assessing the corrosivity potential of chemicals and chemical mixtures?

2. Does the assay adequately consider and incorporate, where scientifically feasible, the 3Rs of animal use (refinement, reduction, and replacement alternatives)? Does the assay offer advantages with respect to animal welfare considerations?

EPISKIN™ skin model was adequate for assigning packing groups according to the EU skin corrosion hazard classes (R34/R35) and the UN packing group classifications (I and II/III). However, since the performance of EPISKIN™ was not assessed for distinguishing between UN packing group II and packing group III, all R34 classifications would be conservatively classified as packing group II.

In response to the second question, EPISKIN™ sufficiently considers and incorporates the 3Rs. Specifically, the use of EPISKIN™ offers advantages with respect to animal welfare considerations, including animal use refinement, reduction, and replacement. Similarly, the use of this assay as part of an integrated approach reduces and refines the use of animals by providing a basis for decisions on further testing. When this method is used as part of the integrated testing strategy for corrosivity/irritation, there is a reduction in the number of animals required because positive results usually eliminate the need for animal testing, and when further testing in animals is determined to be necessary, only one animal could be required to identify a corrosive chemical (one animal is used if the *in vitro* test is negative).